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Affinities of the Madagascan endemic *Geosiris*, *Iridaceae* or *Geosiridaceae*

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Summary : The monotypic Madagascan endemic *Geosiris* is a lilioid monocot, unusual in being an achlorophyllous saprophyte. Its affinities are uncertain but it has been placed in *Burmanniaceae* or *Iridaceae* or treated as a separate family *Geosiridaceae*. Anatomical investigation has shown that *Geosiris* has styloid crystals in the rhizome and flowering stem, and vessels of an unspecialized type that have scalariform perforation plates in the roots and possibly in the flowering stems. The presence of styloid crystals in conjunction with three stamens and an inferior ovary suggest that *Geosiris* belongs in *Iridaceae* and may be closely related to the Afro-Madagascan *Aristea*. Perianth and pollen morphology are consistent with this treatment. Similarly, the presence of the flavonoid quercetin in *Geosiris* supports its placement in *Iridaceae* in which this compound is a frequent chemical constituent, and in *Nivenioideae* where flavonols are the predominant phenolics. Embryological data (RÜBSAMEN, pers. comm.) including successive microsporogenesis and helobial endosperm formation may or may not be consistent with *Iridaceae* in which only simultaneous miscroporogenesis and nuclear endosperm formation have been reported but only in a limited number of relatively specialized genera.

Résumé : Le genre monotypique *Geosiris*, endémique de Madagascar, est une Monocotylédone lilioïde et, fait inhabituel dans ce groupe, un saprophyte sans chlorophylle. Ses affinités sont incertaines, mais il a été placé dans les *Burmanniaceae* ou les *Iridaceae*, ou encore considéré comme une famille distincte. Une étude anatomique a montré que *Geosiris* possède des cristaux styloïdes dans le rhizome et la tige florifère, et des vaisseaux d'un type non spécialisé avec des cloisons à perforations scalariformes dans les racines et probablement aussi dans les tiges florifères. La présence de cristaux styloïdes, conjointement avec trois étamines et un ovaire infère, suggère que *Geosiris* appartient aux *Iridaceae* et peut être proche du genre Afro-malgache *Aristea*. La morphologie du périanthe et du pollen est compatible avec ce point de vue. De même, la présence chez *Geosiris* du flavonoïde quercétine plaide en faveur de son classement parmi les *Iridaceae*, dans lesquelles ce produit est un constituant chimique fréquent, et dans les *Nivenioideae* chez lesquelles les flavonols sont les composés phénoliques prédominants. Des données embryologiques (RÜBSAMEN, comm. pers.) montrant la succession de la microsporogenèse et la formation de l'albumen hélobial peuvent ou non être compatibles avec les *Iridaceae* chez lesquelles seules la simultanéité de la microsporogenèse et la formation de l'albumen nucléaire ont été signalées, mais uniquement dans un petit nombre de genres relativement spécialisés.

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INTRODUCTION

Geosiris is a monotypic genus endemic to mesic areas of the subtropical Indian Ocean island of Madagascar and the nearby Ile Ste. Marie. The sole species, *G. aphylla*, is a small achlorophyllous saprophyte, which has scale-like leaves, swollen corm-like rhizomes, and small blue flowers aggregated at the apices of the flowering stem and branches. When first described by BAILLON in 1894 it was assigned to *Iridaceae* (BAILLON, 1894, 1895 : 152), which it resembles in having three stamens (those of the inner whorl being absent) and an inferior, trilocular ovary with axile, dendroid placentation.

Shortly afterwards, ENGLER (1897 : 96), in a supplement to *Die Natürlichen Pflanzenfamilien*, placed *Geosiris* in *Burmanniaceae*. However, JONKER (1938), who monographed the family, excluded *Geosiris* from *Burmanniaceae* on the grounds that it differed in the aestivation of the tepals. *Geosiris* has imbricate outer and contorted inner tepals, while *Burmanniaceae* have valvate outer and induplicate inner tepals. The presence of only three stamens does not necessarily exclude *Geosiris* from *Burmanniaceae* but the condition accords better with *Iridaceae*. *Burmanniaceae* (not including *Thismiaceae*) have only three stamens which belong to the inner whorl while the three stamens in *Iridaceae* belong to the outer. The unusual so called "dendroid" placentas, actually widely 2-armed (RÜBSAMEN, pers. comm.), accord well with *Burmanniaceae*, but not with *Iridaceae*. In general aspect *Geosiris* resembles *Burmanniaceae* in its saprophytic achlorophyllous habit, while the style and short stigmatic lobes appear to correspond as well with *Burmanniaceae* as *Iridaceae*. The fruit of *Geosiris* has a persistent perianth and style, also found in *Thismiaceae*, a family sometimes included in *Burmanniaceae*, but in *Thismiaceae* the ovary is unilocular with parietal placentas. Tepal aestivation in *Geosiris* corresponds with *Iridaceae*, as does the development of the outermost floral bracts into spathe-like structures enclosing the inflorescence. Both these features suggest an affinity with *Iridaceae* as originally argued by BAILLON.

JONKER (1939) also pointed out that *Geosiris* differs from *Iridaceae* in its dendroid placentas with numerous ovules, and thick scaly rhizome. JONKER emphasized that the capsule of *Geosiris* is crowned by the basal part of the perianth tube, and he described the perianth as circumscissile. These differences as well as the totally different aspect of the plant led JONKER to conclude that *Geosiris* would be treated as a separate family *Geosiridaceae*, close to *Iridaceae* but nonetheless meriting separation.

Modern systematic opinion has varied only a little. *Geosiris* was included by PERRIER (1946) in *Iridaceae* in his treatment in *Flore de Madagascar et des Comores*. DAHLGREN et al. (1985) recognized *Geosiridaceae* but considered it to be allied to *Iridaceae*, where it is placed as a subfamily by THORNE (1983) and TAKHTAJAN (1980). GOLDBLATT et al. (1984) concluded that *Geosiris* should be excluded from *Iridaceae* because it lacked the distinctive styloid crystals that are characteristic of, and diagnostic for, the family. One of us (DORR) has collected ample herbarium and spirit material of *Geosiris*. As a result we have been able to investigate the flavonoids, to check more thoroughly for the presence of styloids, and to examine the xylem for the presence of vessels and their perforation plate structure. The embryology is also being studied by RÜBSAMEN et al. and some of their preliminary observations are considered.

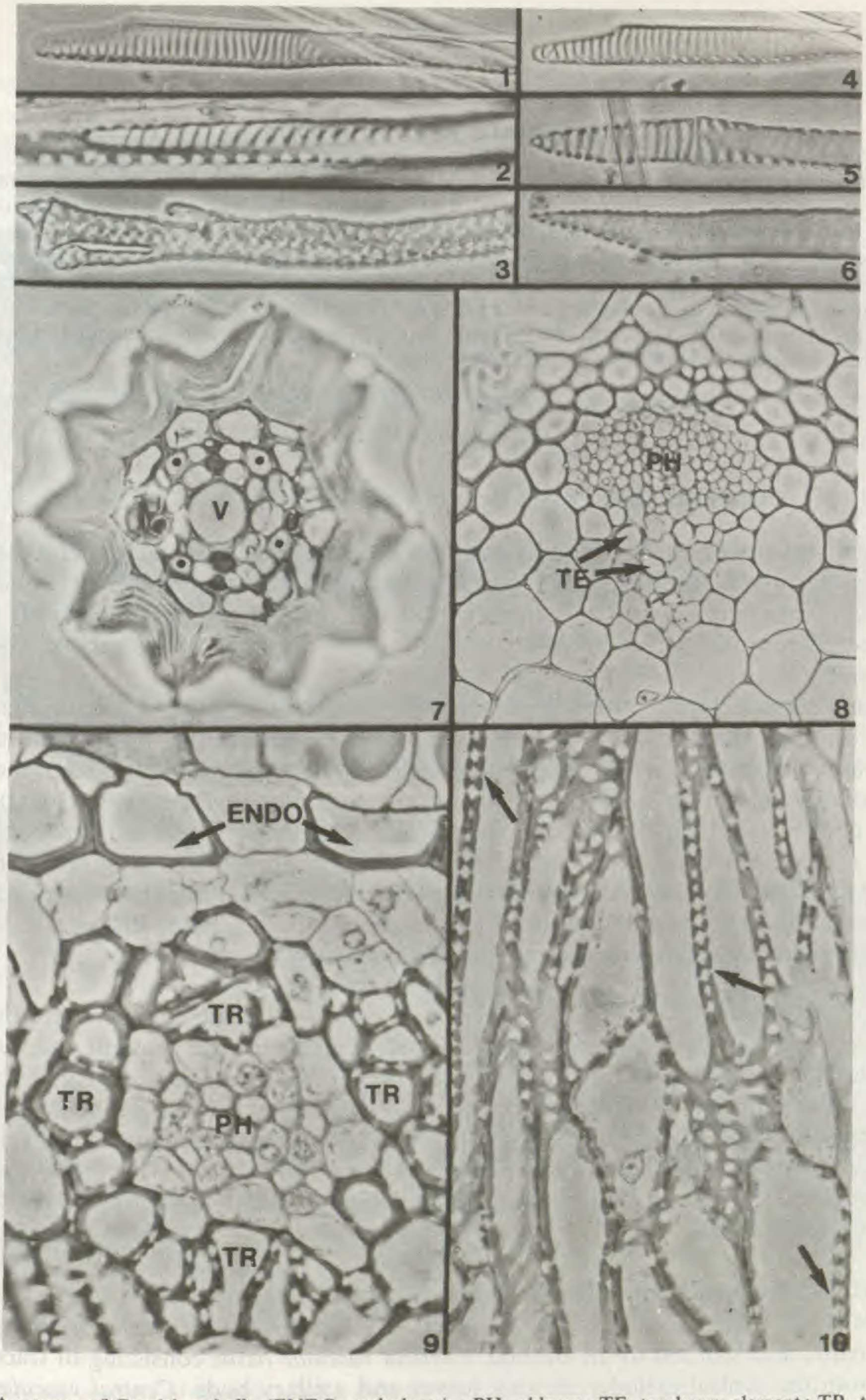


Fig. 1. — Anatomy of **Geosiris aphylla** (ENDO, endodermis; PH, phloem; TE, tracheary element; TR, tracheid; V, central vessel) : **1-6**, parts of separated tracheary elements, probably vessel members except the tracheid in 3; 1 and 4 from the lower flowering stem, probable scalariform perforation plate at two focal levels; 2 and 5 probable perforation plates in two vessel members from the upper part of the flowering stem; 6 profile view of probable scalariform plate in root; **7**, x-section of the stele of a root with central vessel and dots indicating early metaxylem, the thick-walled endodermis not in clear focus; **8**, part of x-section of the upper flowering stem, with phloem, tracheary elements and endodermis indicated; **9**, x-section of rhizome, amphivasal bundle with numerous bordered pits in tracheids; **10**, longi-section of bundle with arrows indicating bordered pit pairs in tracheids. (1, 3, 4, 8 × 445; 2, 5-7, 9, 10 × 715.)

MATERIALS AND METHODS

FAA-fixed samples were gathered in the field from two populations of *Geosiris*. Samples were examined at a) Missouri Botanical Garden, b) Royal Botanic Gardens, Kew, and c) University of California, Santa Barbara. At (a) samples were cleared in household bleach, mounted in glycerin, and viewed between polarizing filters to detect the presence and type of crystals. At (b) samples were sectioned and stained in safranin and alcian blue, dehydrated through an alcohol series and mounted in Euparal. At (c) the samples of all parts except flowers were macerated in acetic acid and hydrogen peroxide, stained in toluidine blue and mounted in glycerin diluted with water. Other samples were embedded in Spurr's resin and sectioned at about 2 μm and stained in toluidine blue.

OBSERVATIONS

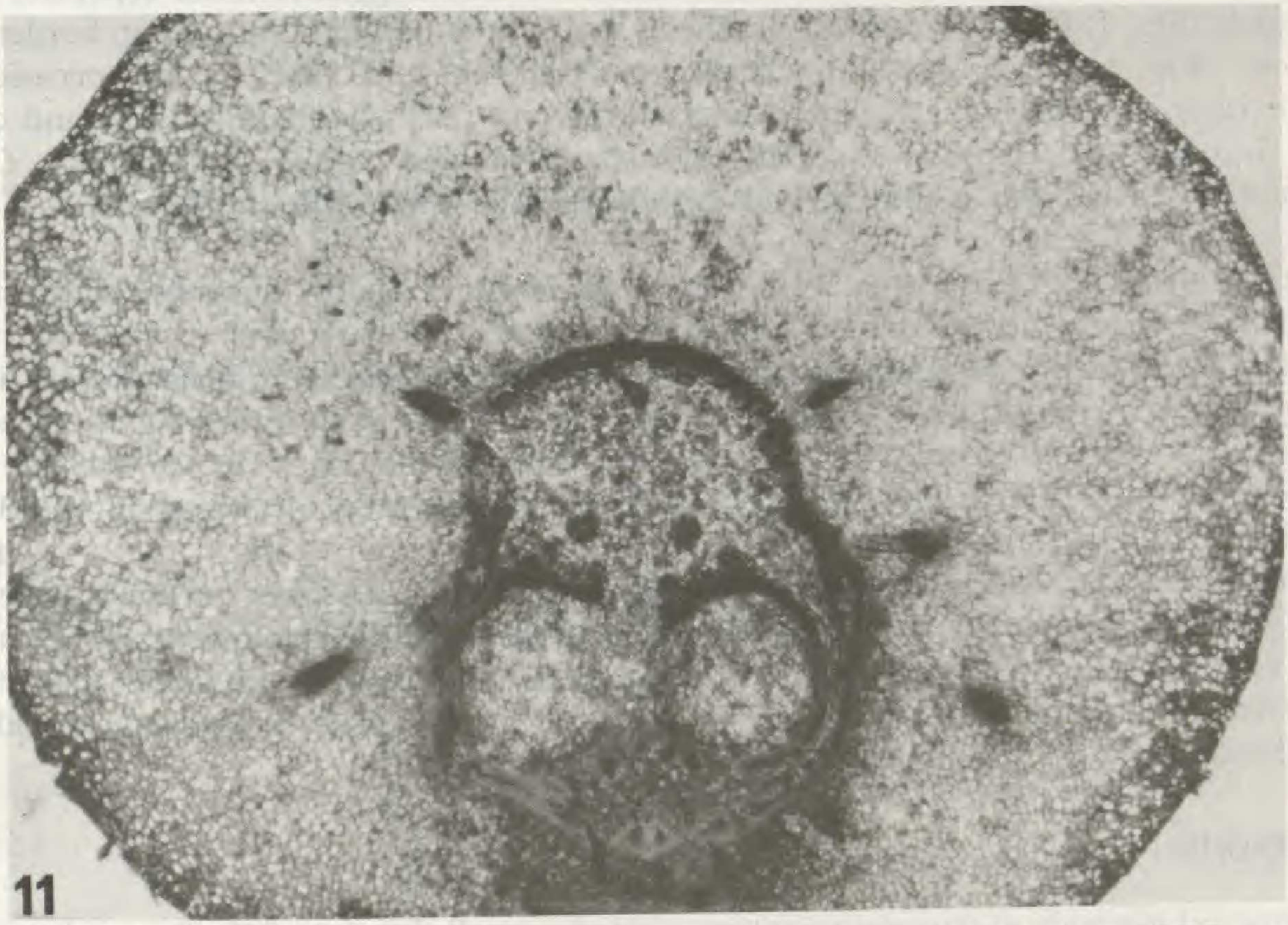
ANATOMICAL OBSERVATIONS

Slender styloids of the type characteristic of the *Iridaceae* (GOLDBLATT et al., 1984) are present in the aerial, flowering stems and are common in the rhizomes. Styloids were previously reported to be absent in *Geosiris* by GOLDBLATT et al. who examined only the scale-like leaves. *Burmanniaceae* lack oxalate crystals but some species of the related *Thismiaceae* have raphides (DAHLGREN et al., 1985; RÜBSAMEN, 1986).

Geosiris has vessels in the roots (Fig. 1, *6*) and possibly also in the flowering stem (Fig. 1, *1, 2, 4, 5*). The perforation plates are unspecialized (CHEADLE, 1953) with numerous scalariform perforations. Similar vessels occur in *Iridaceae* in the roots of *Aristea*, the shrubby Cape genera *Nivenia, Klattia*, and *Witsenia*, and the Australasian *Patersonia* (CHEADLE, 1963; RUDALL, unpubl. obs.), which comprise *Nivenioideae*. Vessels are absent in the shoot system of *Iridaceae* except *Sisyrinchium* and specialized vessels with predominantly simple perforations are found in subfamilies *Iridoideae* and *Ixioideae*. *Burmanniaceae* have vessels with scalariform perforations in both the root and shoot system (DAHLGREN et al., 1985; RÜBSAMEN, 1986). The results of the anatomical investigation are presented in detail below.

ROOT : *Epidermis* thin-walled. *Cortex* narrow, composed of up to 4 layers of thin-walled cells. *Endodermis* a very conspicuous layer of cells with inner and most of the radial walls heavily thickened, U-or V-shaped in x-section (Fig. 1, *7*). *Pericycle* comprising small cells. *Vascular tissue* continuous with peripheral vasculature of central cylinder of rhizome, comprising several alternating strands of xylem and phloem surrounding central thick-walled tissue. *Vessels* present, the perforation plates scalariform (Fig. 1, *7*) with the longest having over 40 bars, the shortest 10; largest vessel diam. 12 μm, smallest 7 μm, longest vessel member 705 μm. *Crystals* absent.

RHIZOME : *Epidermis* thin-walled, lacking stomata. *Periderm* absent. *Cortex* parenchymatous, wide (Fig. 2, *11, 12*), often as wide as central vascular cylinder, cells oriented in radial files internally, disorganized to the outside. *Cortical vascular tissue* consisting of traces leading directly from the central cylinder to scale leaves and axillary buds. *Central vascular cylinder* with an endodermal layer of parenchymatous cells becoming thickened on inner and radial walls in older tissue and continuous with endodermis of roots, vascular tissue immediately



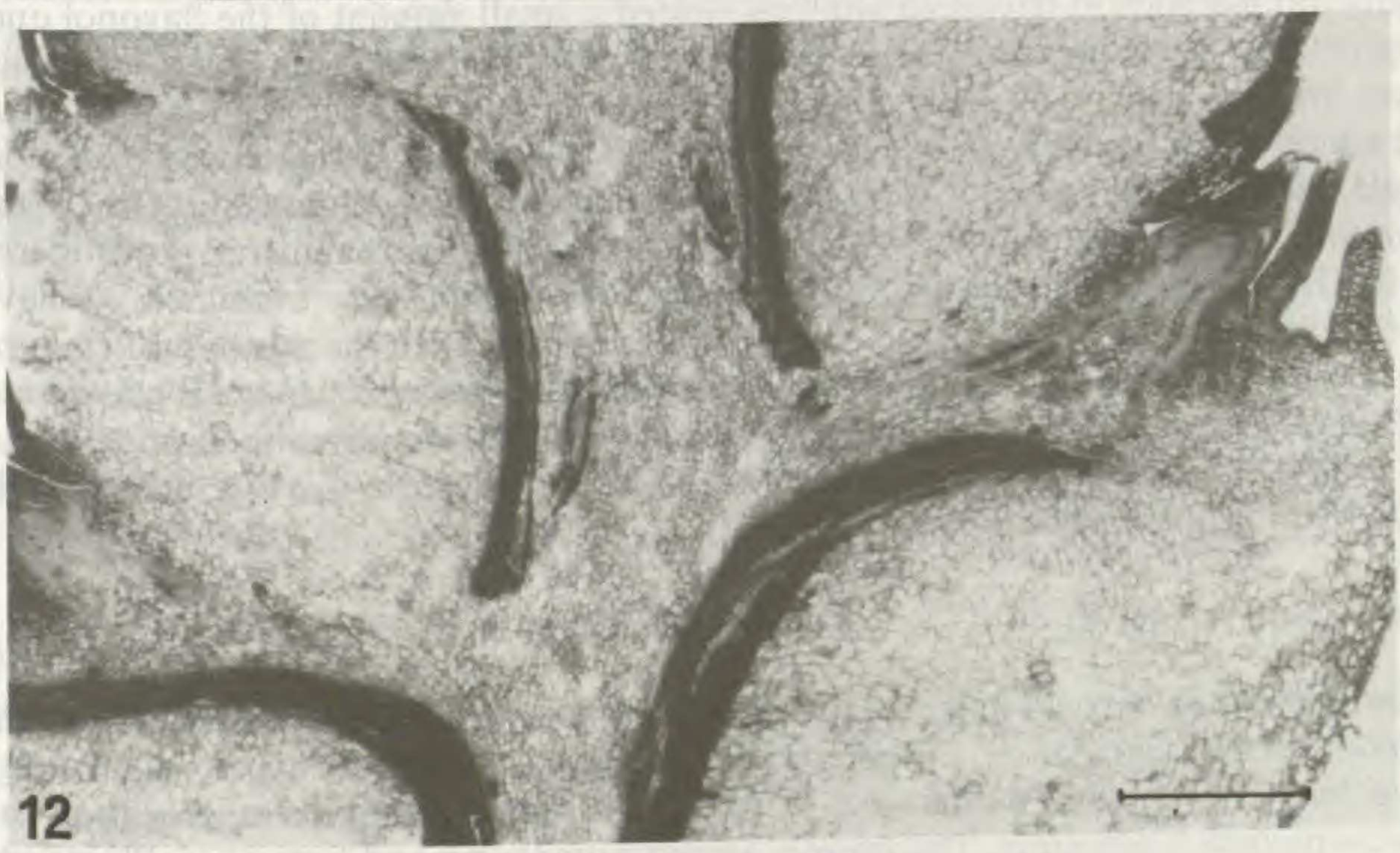


Fig. 2. — Rhizome anatomy of **Geosiris aphylla**, showing the wide cortex and central vascular cylinder with traces leading to the buds : **11**, x-section ; **12**, longi-section, scale line = 1 mm.

within endodermis (Fig. 1, *9*), comprising a cylinder of coalesced bundles with occasional gaps where traces lead to buds and scale leaves; bundles in central ground tissue separate, usually amphivasal (Fig. 1, *9*). *Vessels* absent, tracheids to 14 μm diam., to 656 μm long, bordered pits numerous (Fig. 1, *10*), branched tracheids common (Fig. 1, *3*). *Tannin* cells occasional in ground tissue, especially in epidermis, outer cortex and cells surrounding roots and cortical traces. *Starch* granules present, especially in inner cortex and central ground tissue. *Crystals* present in cortex and pith as short styloids with pointed or square ends, infrequent.

FLOWERING STEM : Circular in cross section. *Epidermis* of slightly thickened walls, stomata infrequent. *Cortex* of up to 10 layers of parenchymatous cells with small intercellular spaces, the outermost layer of cells often with thickened walls. *Sclerenchyma cylinder* present at inner limit of cortex, comprising several to 10 layers (Fig. 1, *8*) of thick-walled lignified cells, the walls thinnest in layer adjacent to the pith. *Pith* parenchymatous. *Vascular bundles* within sclerenchyma cylinder only (absent from cortex and central pith), occurring in 1-2(-3) rings (1 in Fig. 1, *8*), the largest bundles innermost. *Vessels* possibly present, with scalariform perforation plates or scalariform pitting on long end walls in both lower part with scale leaves (Fig. 1, *1, 4*) and in upper part (Fig. 1, *2, 5*). "*Vessel members*" in the lower part up to 16 μm diam., up to 1,476 μm long; in upper part (inflorescence axis) up to 14 μm diam., up to 997 μm long. *Tannin* cells present in cortex and pith. *Crystals* occasionally present as styloids with pointed ends in cells surrounding the sclerenchyma cylinder.

FLAVONOIDS

Standard methods of flavonoid analysis yielded a small amount of the flavonol quercetin, as a diglycoside, in the flowering stem and inflorescence of *Geosiris*. Flavone C-glycosides and flavonols are the most frequent flavonoids in *Iridaceae* (WILLIAMS et al., 1986) and flavovols are the major constituents in *Nivenioideae*. Species of *Aristea* are characterized by the presence of simple quercetin and kaempferol glycosides and the quinone, plumbagin. Other genera of *Nivenioideae* have more complex glycosides of quercetin, isorhamnetin, kaempferol, and myricetin. In *Gymnosiphon cymosus*, only member of *Burmanniaceae* examined, no flavonoids were detected. Thus the flavonoid data are clearly consistent with the position of *Geosiris* close to *Aristea* in *Nivenioideae*. The colored pigment in the blue flowers of *Geosiris* was identified as a delphinidin 3-monoside (probably the 3-glucoside). This simple anthocyanin in a family where complex glycosylation is the rule is also consistent with *Geosiris* being a relatively unspecialized member of *Iridaceae*.

MORPHOLOGICAL NOTES

Observations in the field make it possible to add new details to the description of *Geosiris*. The inflorescence ranges from a single terminal unit consisting of a pair of flowers in an apparent binate rhipidium (WEIMARCK, 1939), to a congested head-like aggregation in which the basic structure is not clear. The flowers are actinomorphic and fugacious. Plants collected in the morning have open flowers but a population found in the afternoon had wilted flowers. The flowers have a strong sweet odor. Unlike the published figures (DAHLGREN et al., 1985) the tepals are not cupped but spread horizontally at right angles to the short tube (Fig. 3). The

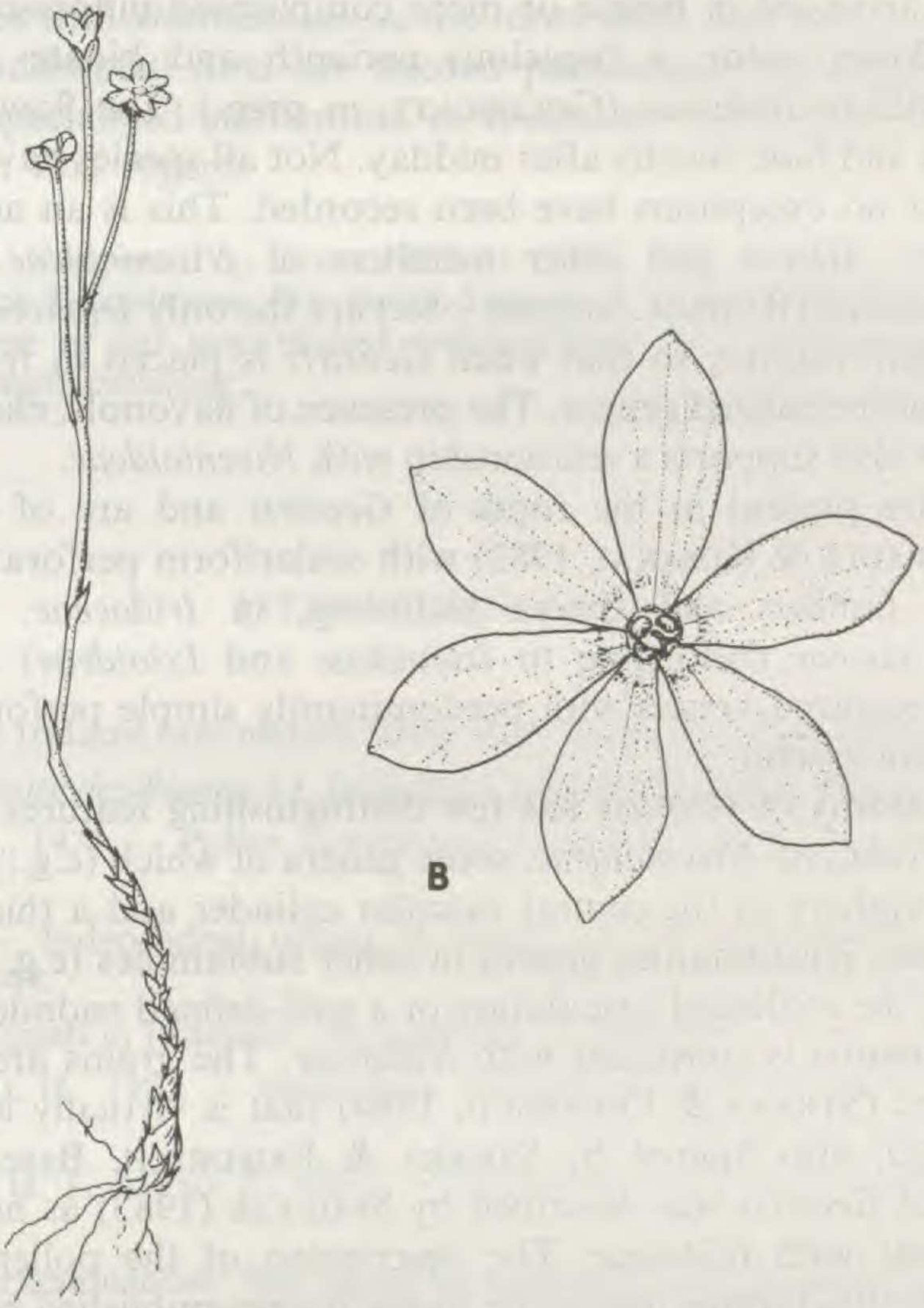


Fig. 3. — Morphology of **Geosiris aphylla** : **A**, whole plant × 0.5; **B**, flowers from above × 4.

perianth color is pale blue-purple, darker at the margins fading to nearly white at the base of the tepals, while the throat is yellow. The tepals are similarly colored on the reverse but have a conspicuous purple midvein, and are sometimes apiculate. The stems are whitish or flushed reddish to purple.

DISCUSSION

The discovery of styloids in *Geosiris* links the genus with *Iridaceae* in which styloids occur in all genera except *Sisyrinchium* (GOLDBLATT et al., 1984) and some of its close allies. The presence of styloids in conjunction with the inferior ovary and three stamens (belonging to the outer whorl of the androecium), makes it seem unreasonable to exclude *Geosiris* any longer from *Iridaceae*. And once its position is accepted here, it is difficult to avoid the conclusion made by BAILLON almost 100 years ago, that *Geosiris* is probably related to the Afro-Madagascan *Aristea* (ca. 50 spp.). *Aristea* has fugacious blue to blue-purple flowers with a

short perianth tube, arranged in binate or more complicated inflorescence units (WEIMARCK, 1939, 1940). Blue flower color, a fugacious perianth and binate inflorescence units are probably derived states in *Iridaceae* (GOLDBLATT, in prep.). The flowers of *Aristea* typically open in the mornings and fade shortly after midday. Not all species have been examined in this connection but so far no exceptions have been recorded. This is an added feature shared by *Aristea* and *Geosiris*. *Aristea* and other members of *Nivenioideae* and *Isophysis* of the monotypic *Isophysidoideae* (RUDALL, unpubl. obs.) are the only *Iridaceae* recorded with vessels having scalariform perforations, so that when *Geosiris* is placed in *Iridaceae*, it is consistent with these relatively unspecialized genera. The presence of flavonols, characteristic constituents of *Aristea*, in *Geosiris* also supports a relationship with *Nivenioideae*.

Xylem vessels are present in the roots of *Geosiris* and are of the unspecialized type (CHEADLE, 1953; CHEADLE & KOSAKAI, 1982) with scalariform perforation plates as described for many monocot families and genera including, in *Iridaceae, Aristea, Nivenia,* and *Patersonia*. Other *Iridaceae* (belonging to *Iridoideae* and *Ixioideae*) examined by CHEADLE (1963) have more specialized vessels with predominantly simple perforations. Most *Iridaceae* lack vessels in the shoot system.

The rhizome anatomy of *Geosiris* has few distinguishing features but is not inconsistent with its inclusion in *Iridaceae-Nivenioideae*, some genera of which (e.g., *Aristea*) have coalesced vasculature at the periphery of the central vascular cylinder and a thick-walled endodermoid layer in older rhizomes. Rhizomatous genera in other subfamilies (e.g., *Iris, Dietes-Iridoideae*) frequently lack either the coalesced vasculature or a well-defined endodermis (RUDALL, 1984).

The pollen of *Geosiris* is consistent with *Iridaceae*. The grains are ellipsoid, sulcate, and have a reticulate exine (STRAKA & FRIEDRICH, 1984) that is virtually identical with that in at least *Aristea kitchingii*, also figured by STRAKA & FRIEDRICH. Based on light microscope observations pollen of *Geosiris* was described by SCHULZE (1983) as having a reticulate exine and as such consistent with *Iridaceae*. The description of the pollen grains of *Geosiris* as sulcoidate (ERDTMAN, 1952) seems inaccurate as the figures published by STRAKA & FRIEDRICH and by SCHULZE show a typical sulcate grain. *Burmanniaceae* are reported to have sulcate or 1-2-porate pollen grains and psilate exine (CHAKRAPANI & RAJ, 1971; ZAVADA, 1983) but a few *Thismiaceae* (*Burmanniaceae-Thismieae*) have a type of reticulate exine (RÜBSAMEN, 1986 : 105).

The embryology of *Geosiris*, until now unknown, is currently being studied by T. RÜBSAMEN and colleagues. Their preliminary observations (RÜBSAMEN, pers. comm.) indicate that *Geosiris* has successive microsporogenesis and helobial endosperm formation, two features that have not been reported in *Iridaceae* but are characteristic of *Burmanniaceae*. However, a parietal cell is produced which is characteristic of *Iridaceae* and not of *Burmanniaceae* although a parietal cell is also produced in the closely allied *Corsiaceae* (RÜBSAMEN, 1986 : 167). The apparent discordance with *Iridaceae* may not be significant for few *Iridaceae* are known embryologically. Nuclear endosperm formation is recorded for *Iris, Belamcanda*, and *Sisyrinchium* (*Iridoideae*) and a few specialized genera of *Ixioideae* including *Crocus* and *Romulea*. Simultaneous microsporogenesis, regarded as characteristic of *Iridaceae* has actually been reported in few genera, none belonging to *Nivenioideae*.

The relationships of *Geosiridaceae* remain uncertain but we conclude that the weight of evidence at hand warrants the inclusion of *Geosiris* in *Iridaceae* subfamily *Nivenioideae*. The

similarities that it shares with *Burmanniaceae* are most likely due to convergence for a similar life form and habit. Additional data are needed particularly concerning the nature of the embryology of the unspecialized subfamilies of *Iridaceae*, *Nivenioideae* and *Isophysidoideae*, which are unknown in this regard.

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LITERATURE CITED

BAILLON, H., 1894. — Une Iridacée sans matière verte. *Bull. Mens. Soc. Linn. Paris* 2 (146) : 1149-1150.

BAILLON, H., 1895. — *Histoire des Plantes* 13. Iridacées : 118-164. Hachette, Paris.

CHAKRAPANI, P. & RAJ, B., 1971. — Pollen morphological studies in the Burmanniaceae. *Grana* 11 : 164-179.

CHEADLE, V. I., 1953. — Independent origin of vessels in the monocotyledons and dicotyledons. *Phytomorph.* 3 : 23-44.

CHEADLE, V. I., 1963. — Vessels in Iridaceae. *Phytomorph.* 13 : 245-248.

CHEADLE, V. J. & KOSAKAI, H., 1982. — Occurrence and specialization of vessels in Xyridales. *Nordic J. Bot.* 2 : 97-109.

DAHLGREN, R., CLIFFORD, H. T. & YEO, P., 1985. — *The families of the Monocotyledons*. Springer-Verlag, Heidelberg.

ENGLER, A., 1897. — Burmanniaceae. ?6a. Geosiris. *In* ENGLER & PRANTL (editors), *Die Natürlichen Pflanzenfamilien*, Nachtrag und Register zu Teil 2-4 [1] : 96. Engelmann, Leipzig.

ERDTMAN, G., 1952. — *Pollen Morphology and Plant taxonomy. Angiosperms*. Almqvist and Wiksell, Stockholm.

GOLDBLATT, P., HENRICH, J. E. & RUDALL, P., 1984. — Occurrence of crystals in Iridaceae and allied families and their phylogenetic significance. *Ann. Missouri Bot. Gard.* 71 : 1013-1020.

JONKER, F. P., 1938. — A monograph of Burmanniaceae. *Meded. Bot. Mus. Herb. Rijks Univ. Utrecht* 51 : 1-279.

JONKER, F. P., 1939. — Les Géosiridacées, une nouvelle famille de Madagascar. *Recueil Trav. Bot. Néerl.* 36 : 473-479.

PERRIER DE LA BÂTHIE, H., 1946. — Iridacées. *In* HUMBERT (editor), *Flore de Madagascar et des Comores* 45 : 1-41. Imprimerie Officielle, Tananarive.

RÜBSAMEN, T., 1986. — Morphologische, embryologische und systematische Untersuchungen an Burmanniaceae und Corsiaceae (Mit Ausblick auf die Orchidaceae-Apostasioideae). *Diss. Bot.* 92 : 1-310.

RUDALL, P., 1984. — Taxonomic and evolutionary implications of rhizome structure and secondary thickening in Iridaceae. *Bot. Gaz.* 145 : 524-534.

SCHULZE, W., 1971. — Beiträge zur Pollenmorphologie der Iridaceae und ihre Bedeutung für die Taxonomie. *Feddes Rep.* 82 : 101-124.

SCHULZE, W., 1983. — Beiträge zur Taxonomie der Liliifloren. XIII. Hewardiaceae und Geosiridaceae. *Wiss. Z. Friedrich-Schiller-Univ. Jena, Math.-Naturwiss. R.* 32 : 981-984.

STRAKA, H. & FRIEDRICH, B., 1984. — Palynologia Madagassica et Mascarenica. Gymnospermae und Monocotyledones. *Trop. & Subtrop. Pflanzenwelt* 49 : 1-89.

TAKHTAJAN, A., 1980. — Outline of the classification of flowering plants (Magnoliophyta). *Bot. Rev.* 46 : 225-359.

THORNE, R., 1983. — Proposed new realignments in the angiosperms. *Nordic J. Bot.* 3 : 85-117.

WEIMARCK, H., 1939. — Types of inflorescences in Aristea and some allied genera. *Bot. Not.* : 616-626.

WEIMARCK, H., 1940. — Monograph of the genus Aristea. *Lunds Univ. Arssk N. F. Avd.* 2 (36) : 1-140.

WILLIAMS, C. A., HARBONE, J. B. & GOLDBLATT, P., 1986. — Correlations between phenolic patterns and tribal classification in the family Iridaceae. *Phytochem.* 25 : 2135-2154.

ZAVADA, M. S., 1983. — Comparative morphology of monocot pollen and evolutionary trends of apertures and wall structures. *Bot. Rev.* 49 : 331-379.